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ZENECA Inc.  
Wilmington, DE 19897

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**Study Title**

Enforcement Method for the Determination of Residues  
of ICIA5504 and R230310 in Grain and Grapes  
for EPA Confirmation

*Shaw*

**Data Requirement**

Subdivision O  
Guideline Ref. 171-4 (c)

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**Study Completed On**

May 1995

**Performing Laboratory**

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**Laboratory Project ID**

RAM 243/04

Study Number : RAM 243/04  
Report Title : Enforcement Method for the Determination of Residues  
of ICIA5504 and R230310 in Grain and Grapes  
for EPA Confirmation

**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

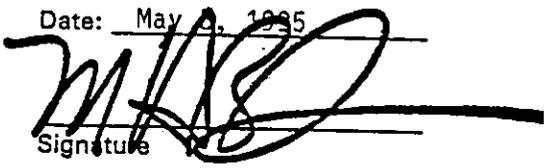
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Sections 510(d)(1)(A), (B) or (C).

Company : ZENECA Inc.

Company Agent : Michele A. Beguhn

Date: May 4, 1995

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Title

  
Signature

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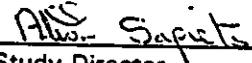
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of ICIA5504 and R230310 in Grain and Grapes  
for EPA Confirmation

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

This study has been conducted in compliance with the Principles of Good Laboratory Practice (GLP) laid down in the United Kingdom Department of Health Compliance Programme (1989).

Under the Memorandum of Understanding signed by both the United States of America and the United Kingdom this study is considered to satisfy the requirement that it be conducted in accordance with 40 CFR Part 160.

  
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## STUDY TITLE

Enforcement Method for the Determination of Residues of ICIA5504 and R230310 in Grain and Grapes for EPA Confirmation

## SUMMARY

Comments provided by the independent laboratory suggested the following upgrades to the enforcement method:

1. Example chromatograms should be included in the SOP. An indication of the expected retention times for ICIA5504 and R230310 with columns other than the Rtx-200 capillary columns for use when establishing the gas chromatography before sample analysis. Now found in Appendix 1 and 2.
2. Details of the partition of the extract with dichloromethane should be more specific. Section 3.2.d now contains specific instructions with shaking times.

This method is appropriate as the enforcement method for determining residues of ICIA5504 and R230310 in grapes and grape processed commodities.

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**RAM 243 : EPA ENFORCEMENT METHOD**

**RESIDUE ANALYTICAL METHOD FOR THE ANALYSIS OF ICIA5504 AND R230310 IN  
CEREALS (GRAIN) AND VINES (GRAPES).**

**Authors: S R Burke & A Sapiets Issuing Section: Residue Chemistry**

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## 1 SCOPE

The analytical procedures described are suitable for the determination of residues of the fungicide ICIA5504, Figure 1, and its geometrical isomer, R230310 (Figure 2) in cereals (grain) and vines (grapes).

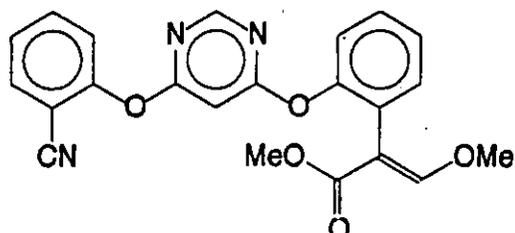


Figure 1: Methyl (E)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl]-3-methoxyacrylate (IUPAC).

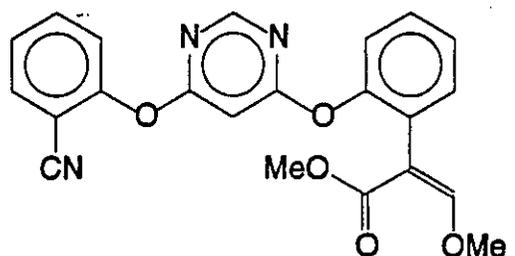


Figure 2: Methyl (Z)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl]-3-methoxyacrylate (IUPAC).

Using this method, a batch of ten samples plus two procedural recovery samples can be analysed within a 24 hour period.

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## 2 SUMMARY

ICIA5504 and R230310 residues in grain samples are extracted in 90:10/ acetonitrile:water. An aliquot of the extract is cleaned up by adsorption chromatography on a Florisil column. The eluate is evaporated to dryness and taken up in a known volume of acetone for analysis by gas chromatography with nitrogen-phosphorus detection (GC-NPD).

ICIA5504 and R230310 residues in grape samples are extracted in 90:10/ acetonitrile:water. An aliquot of the extract is cleaned up by adsorption chromatography on a silica sorbent. The eluate is evaporated to dryness and taken up in a known volume of acetone for analysis by GC-NPD or in mobile phase for analysis by high performance liquid chromatography with ultra-violet detection (HPLC-UV).

## 3 PROCEDURE

### 3.1 Sample Preparation

Samples should be removed from the deep freeze and allowed to stand at room temperature for approximately 30 minutes. Samples should be minced/chopped until a truly homogenous sample is obtained.

Grape samples which are removed from the deep freeze having previously been homogenised, should be allowed to thaw for the minimum period only before breaking up and weighing out; this ensures that no partition of the endogenous water content can occur prior to analysis.

### 3.2 Extraction

- a) Thoroughly mix the sample and weigh a representative aliquot (20 g) into a centrifuge bottle.
- b) Grain: If the samples are very dry, pre-wet with acetonitrile (~10 cm<sup>3</sup>) and soak for 15 minutes prior to fortification. Fortify two control samples with an accurately known amount of ICIA5504 and R230310 as recovery checks.  
  
Grape: Fortify two control samples with an accurately known amount of ICIA5504 and R230310 as recovery checks.
- c) Grain and grape: Homogenise for two minutes in 90:10/acetonitrile:water (grapes: 60 cm<sup>3</sup>, grain : additional 50 cm<sup>3</sup>). Filter the extract under vacuum through a Whatman No. 1 filter paper into a round bottom flask. Rinse the residuum with further extraction solvent. Adjust to a suitable known volume (eg. 100 cm<sup>3</sup>) with acetonitrile.
- d) Grain - Take a funnel, plug with glass wool and add anhydrous sodium sulphate (~10 g). Pre wet with ethyl acetate (~10 cm<sup>3</sup>), pass an aliquot (2 g) through and rinse with ethyl acetate (20 cm<sup>3</sup>), collecting in a round bottom flask.

Grape - Take a 1 g aliquot and partition with an equivalent volume of dichloromethane plus half equivalent-volume of 5% sodium chloride solution in a separatory funnel. Shake the separatory funnel for about 60 seconds. Collect the dichloromethane layer through anhydrous sodium sulphate, into a round bottom flask. Rinse the sodium sulphate plug with further dichloromethane (~5 cm<sup>3</sup>), collecting in the round bottom flask.

If emulsions occur a second partition with dichloromethane may be required.

- e) Evaporate the aliquots to dryness on a rotary evaporator at  $\leq 40^{\circ}\text{C}$  and:

Grain: Redissolve in 20% ethyl acetate:hexane ( $2\text{ cm}^3$ ) for Florisil column clean-up and ultrasonicate.

Grape: Redissolve in 50:50/hexane:dichloromethane ( $2\text{ cm}^3$ ) for silica column clean-up and ultrasonicate.

### 3.3 Solid Phase Extraction Clean-up (Silica, Si)

- a) Grape samples are cleaned up on a silica column.

Place a disposable silica (Si, 0.5 g) cartridge in a Supelco<sup>™</sup> vacuum manifold assembly. Add 50:50/hexane:dichloromethane ( $3\text{ cm}^3$ ), allow to drip under gravity.

- b) Transfer the sample extract from Section 3.2 (e) above onto the column, and allow to drip under gravity.
- c) Wash the round bottom flask that contained the aliquot with 95:5/dichloromethane:ethyl acetate ( $2\text{ cm}^3$ ) and load onto the cartridge. Allow to drip under gravity. Elute the cartridge with 70:30/dichloromethane:ethyl acetate ( $4\text{ cm}^3$ ), allowing to drip under gravity then dry the column by pushing the eluate through under positive pressure into the collection tubes.
- d) Evaporate the eluates to dryness at  $\leq 40^{\circ}\text{C}$  under a stream of clean dry air. Redissolve the residuum in a known volume of acetone for analysis by GC-NPD or in mobile phase for analysis by HPLC-UV.
- e) Standards used for HPLC-UV analysis should be prepared by pipetting the required amount e.g.  $2\text{ cm}^3$  of a  $0.1\text{ }\mu\text{g cm}^{-3}$  standard, into an HPLC vial, blowing to dryness and taking up in mobile phase (e.g.  $2\text{ cm}^3$  to give a  $0.1\text{ }\mu\text{g cm}^{-3}$  standard concentration).

Prior to use, each fresh batch of solid phase extraction cartridges should be calibrated for the crop to be analysed.

### 3.4 Adsorption Column Chromatographic Clean-up (Florisil<sup>™</sup>)

- a) Grain samples are cleaned up on a Florisil<sup>™</sup> column.

- b) Preparation of Florisil<sup>™</sup> (5% water deactivated) columns

Prepare 5% water deactivated Florisil<sup>™</sup> by drying Florisil<sup>™</sup> in an oven at  $110^{\circ}\text{C}$  for 24 hours, cool and weigh into 100 g batches. Add  $1\text{ cm}^3$  of ultra pure water, place a glass rod in with the Florisil<sup>™</sup> and tumble for one hour. Repeat until  $5\text{ cm}^3$  has been added to give 5% water deactivated Florisil<sup>™</sup>.

Place a small glass wool plug in the bottom of a 10 mm diameter chromatography column (pre-rinsed with acetone and hexane) and add n-hexane ( $15\text{ cm}^3$ ). Slowly, with gentle tapping, add 5% water deactivated Florisil<sup>™</sup> (2 g) followed by granular anhydrous sodium sulphate (1 g). Allow the hexane to percolate onto the column.

**Note** - Prior to use, each batch of column packing material must be calibrated for the crop to be analysed, as follows: Fortify a control sample aliquot with a mixed ICIA5504 and R230310 standard solution in acetone, such that the concentration of each is  $0.1 \mu\text{g cm}^{-3}$ . Evaporate to dryness and redissolve in 20:80/ethyl acetate:hexane ( $2 \text{ cm}^3$ ), ultrasonicate. Transfer the aliquot to the top of the column and allow it to percolate onto the column. Wash with 20:80/ethyl acetate:hexane ( $20 \text{ cm}^3$ ). Then elute with 70:30/ethyl acetate:hexane and collect three fractions ( $10 \text{ cm}^3$ ) and one fraction ( $5 \text{ cm}^3$ ) of the eluate. Analyse the fractions by GC-NPD to determine the elution pattern.

- c) Transfer the sample extract from Section 3.2 (e) above and allow to percolate onto the column. Elute the column using the procedure determined from the column calibration. Collect the ethyl acetate:hexane eluate in a round bottom flask.
- d) Evaporate the eluates to dryness on a rotary evaporator at  $\leq 40^\circ\text{C}$ . Redissolve the residuum in acetone to give a final concentration of  $2 \text{ g cm}^{-3}$  (grain) and transfer to GC vials for analysis by GC-NPD.

#### 4 GAS CHROMATOGRAPHY WITH NITROGEN PHOSPHORUS DETECTION (GC-NPD)

The conditions for the analysis by GC-NPD will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe optimum use. The following conditions have been found to be satisfactory using a Varian 3400 series gas chromatograph fitted with a Varian 8100 series autosampler:

##### 4.1 GC-NPD Conditions

- (i) Columns:  $R_{t_x} - 1$  (100% dimethyl polysiloxane, OV-1, DB-1, RSL-150 equivalent) fused silica wall coated open tubular megabore  $15 \text{ m} \times 0.53 \text{ mm}$  internal diameter ( $1 \mu\text{m}$  film thickness).
- $R_{t_x} - 200$  (trifluoropropylmethyl polysiloxane), fused silica wall coated open tubular megabore  $30 \text{ m} \times 0.53 \text{ mm}$  internal diameter ( $1 \mu\text{m}$  film thickness) or capillary  $15 \text{ m} \times 0.32 \text{ mm}$  ( $0.5 \mu\text{m}$  film thickness).
- (ii) Oven temperatures:  $R_{t_x} - 1$  :  $70^\circ\text{C}$  (hold 1 minute); program at  $30^\circ\text{C minute}^{-1}$  to  $235^\circ\text{C}$  (hold 5 minutes); program at  $2^\circ\text{C minute}^{-1}$  to  $240^\circ\text{C}$  (hold 8 minutes); program at  $50^\circ\text{C minute}^{-1}$  to  $270^\circ\text{C}$  (hold 10 minutes).
- $R_{t_x} - 200$  : (megabore)  $70^\circ\text{C}$  (hold 1 minute); program at  $20^\circ\text{C minute}^{-1}$  to  $220^\circ\text{C}$ ; program at  $10^\circ\text{C minute}^{-1}$  to  $260^\circ\text{C}$  (hold 15 minutes); program at  $30^\circ\text{C minute}^{-1}$  to  $280^\circ\text{C}$  (hold 10 minutes).
- $R_{t_x} - 200$  : (capillary)  $70^\circ\text{C}$  (hold 1 minute); program at  $30^\circ\text{C minute}^{-1}$  to  $220^\circ\text{C}$ ; program at  $15^\circ\text{C minute}^{-1}$  to  $320^\circ\text{C}$  (hold 2 minutes). These conditions may need to be adjusted according to crop type.
- (iii) Injector:  $R_{t_x} - 1$  and  $R_{t_x} - 200$  (megabore) : Septum programmable injector (SPI):  $40^\circ\text{C}$  (hold 0.1 minute); program at  $150^\circ\text{C minute}^{-1}$  to  $200^\circ\text{C}$  (hold 3 minutes); program at  $150^\circ\text{C minute}^{-1}$  to  $250^\circ\text{C}$  (hold 33 minutes). Injection volume =  $4 \mu\text{l}$ .

Rt<sub>x</sub> - 200 (capillary) : Septum programmable injector (SPI): 40°C (hold 0.1 minute); program at 150°C minute<sup>-1</sup> to 320°C (hold 15 minutes). Injection volume = 2 µl.

- (iv) Gas Flow Rates: Helium (carrier) : 5 cm<sup>3</sup> min<sup>-1</sup>  
 Helium (makeup): 25 cm<sup>3</sup> min<sup>-1</sup>  
 Air: 175 cm<sup>3</sup> min<sup>-1</sup>  
 Hydrogen: 4.5 cm<sup>3</sup> min<sup>-1</sup>
- (v) Detector: Temperature at 300°C or 320°C for Rt<sub>x</sub> - 200 (capillary column).  
 Bead setting: 3.0 - 3.3 amps (depending on condition of bead).  
 Attenuation: 4. Range: 12

Under these conditions, for Rt<sub>x</sub> - 200 capillary column, the retention times of ICIA5504 and R230310 were approximately 12.9 and 13.2 minutes respectively; for Rt<sub>x</sub> - 1 megabore column, approximately 33.1 and 33.8 minutes respectively.

**Note:** These conditions should be adhered to as closely as possible. Conversion of the isomers (R230310 -> ICIA5504) has been seen to occur under lower carrier gas flow rates at high temperatures.

## 5 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING UV DETECTION (HPLC-UV)

The conditions for the analysis by HPLC-UV will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe optimum use. The following conditions have been found to be satisfactory using the instruments detailed below.

### 5.1 HPLC-UV Conditions

- a) Instrument : HPLC fitted with a UV detector e.g Severn 6500 series, pump e.g Waters 501 series, autosampler and integrator or data handling system.
- b) Column : Spherisorb 5 ODS2 column 25 cm x 4.6 mm internal diameter.
- c) Mobile Phase : Acetonitrile/water (55:45 - 60:40) flowing at 1 cm<sup>3</sup> minute<sup>-1</sup>, depending on interfering peaks.
- d) Injector : Waters WISP 712 series (100 µl injection volume).
- e) Detector : 255 nm

Under these conditions, using acetonitrile/water (57:43), the retention time of ICIA5504 and R230310 are 8.3 and 6.8 minutes respectively.

## 6 CALCULATION OF ICIA5504 AND R230310 RESIDUE RESULTS

- a) Make repeated injections of a standard solution containing ICIA5504 and R230310 at 0.1 µg cm<sup>-3</sup> into the GC or HPLC operated under the conditions described in 4.1 and 5.1 above. When a consistent response is obtained measure the peak heights or areas obtained for the standard.

- b) Make an injection of each sample solution and measure the peak height or area of the peaks corresponding to ICIA5504 and R230310.
- c) Re-inject the standard solution after a maximum of four injections of sample solutions.
- d) Calculate the residue in the sample, expressed as  $\text{mg kg}^{-1}$  by proportionation of the ICIA5504 or R230310 peak height or peak area measured for the sample against that for the analytical standard solution.

$$\text{Residue} = \frac{\text{peak height/area in sample}}{\text{peak height/area in standard}} \times \frac{\text{concentration of standard}}{\text{concentration of sample solution}} \times \frac{\text{volume injected (std)}}{\text{volume injected (sample)}}$$

$$= \frac{\text{response}}{\text{response}} \times \frac{\mu\text{g cm}^{-3}}{\text{g cm}^{-3}} \times \frac{\mu\text{L}}{\mu\text{L}} = \mu\text{g g}^{-1} = \text{mg kg}^{-1}$$

## 7 CONTROL AND RECOVERY EXPERIMENTS

At least one untreated sample must be analysed alongside any set of samples, using exactly the same method. This ensures that no unobserved contamination of the samples occurred prior to, or during, the analysis. At least two control samples, accurately fortified with a suitable known amount of ICIA5504 and R230310, should be analysed alongside every batch of treated samples. Fortification amounts should be based on anticipated residue levels. When no residues are expected, the recoveries should be fortified at low levels, typically  $0.02\text{-}0.05 \text{ mg kg}^{-1}$ .

## 8 LIMIT OF DETERMINATION (QUANTITATION)

The limit of determination of the method can be assessed by carrying out recovery experiments at low levels of fortification ( $0.02 - 0.05 \text{ mg kg}^{-1}$ ). Care must be taken when working at the limit of determination to minimise the risk of contamination.

## 9 LIMIT OF DETECTION

The limit of detection of ICIA5504 and R230310 can be investigated by fortifying control crop extracts that have been taken through the analytical method. The limit of detection is defined as the lowest concentration that gives a response  $\geq 4$  times background noise.

## 10 EXAMPLES OF TYPICAL CHROMATOGRAMS - see Appendices 1 and 2

APPENDIX 1

Typical Gas Chromatograms for ICIA5504 and R230310  
Residue Determination in Grain

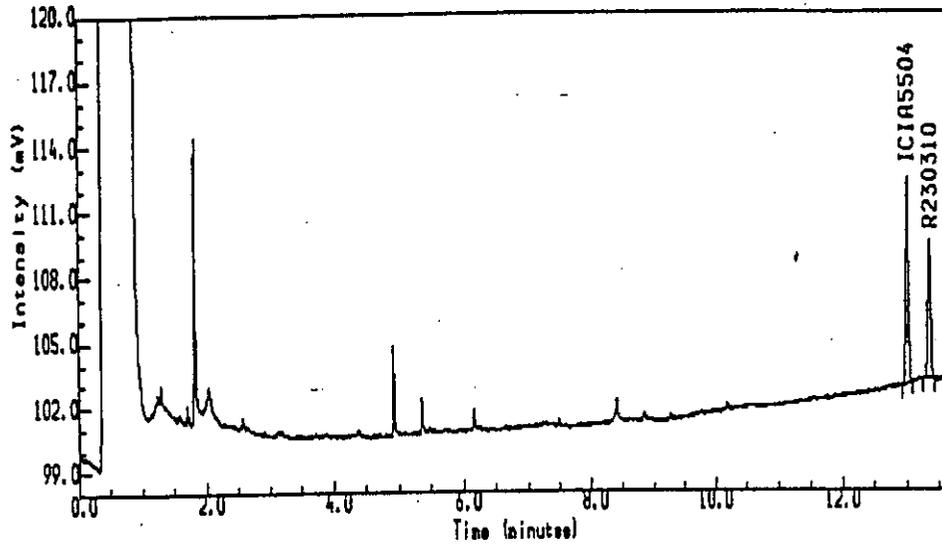
- Figure 1:  $0.1 \mu\text{g cm}^{-3}$  ICIA5504 and R230310 standard.
- Figure 2: Untreated grain sample at  $1.0 \text{ g cm}^{-3}$ .
- Figure 3: Untreated grain sample at  $1.0 \text{ g cm}^{-3}$  fortified at  $0.1 \text{ mg kg}^{-1}$ .  
Recovery = 102% ICIA5504, 98% R230310.

Figure 1

[RESIDUE] 16 AS6949A,1,1  
 Reported on 16-MAY-1995 at 15:30

Injection Report

Acquired on 27-APR-1995 at 18:07



Sample Name : D9579/32W  
 Sample Id : 6949/95/1  
 Sample Type : Standard Amount=1.00000  
 Bottle No. : 1

PEAK INFORMATION

Peak	RT mins	Hgt W	Area U/S	MG/KG	Peak name	Width
1	13.008	9517	26924	0.100	IC1A5504	3.2
2	13.344	6379	19657	0.099	R230310	3.2
<b>Totals</b>						
Unknowns		0	0	0.000		
Named		15896	46581	0.199		
Grand Total		15896	46581	0.199		

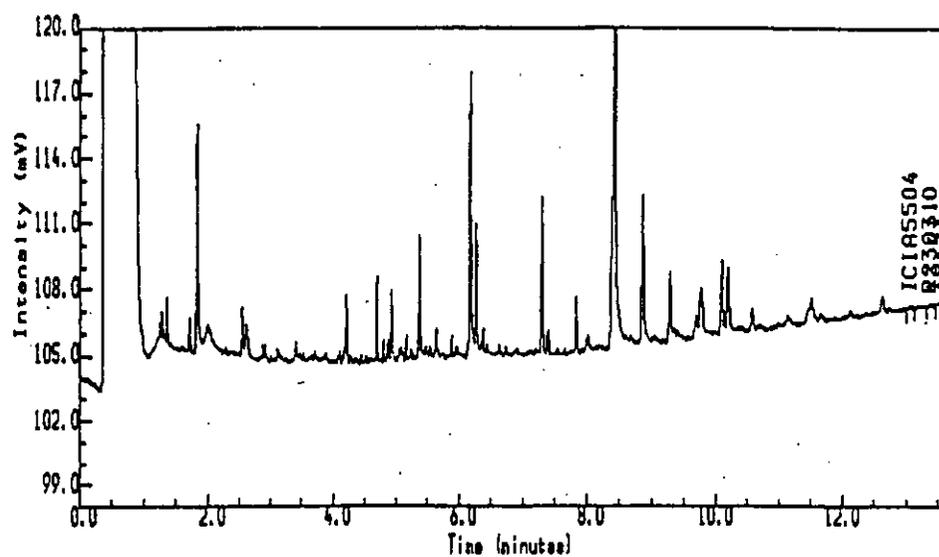
Figure 2

[RESIDUE] 16 AS6949A,2,1  
 Reported on 16-MAY-1995 at 15:31

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 Injection Report

Acquired on 27-APR-1995 at 18:31



Sample Name : 442/1 95  
 Sample Id : 6949/95/2  
 Sample Type : Control Amount=1.00000  
 Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Hght uV	Area uVs	MG/G	Peak name	Width
1	13.024	139	239	0.001	IC185504	1.6
2	13.333	87	74	0.001	R230310	1.3
<u>Totals</u>						
Unknowns		73	100	0.000		
Named		225	313	0.003		
Grand Total		298	413	0.003		

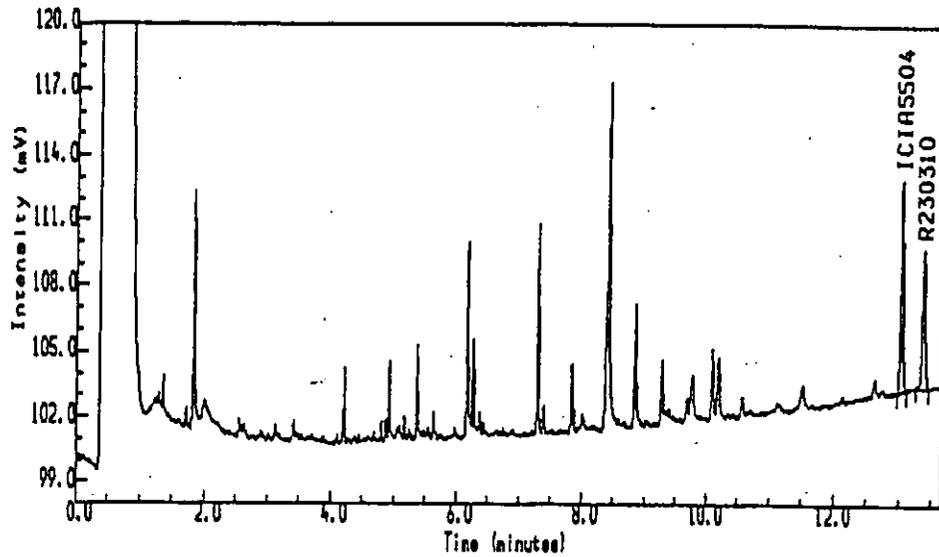
Figure 3

[RESIDUE] 16 AS6949A,3,1  
 Reported on 16-MAY-1995 at 15:31

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 Injection Report

Acquired on 27-APR-1995 at 18:56



Sample Name : R1 442/2 95  
 Sample Id : 6949/95/3  
 Sample Type : Recovery Amount=1.00000  
 Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Hgt uV	Area uVs	MG/AG	Peak name	Width
1	13.029	9710	26506	0.102	IC1A5504	2.9
2	13.365	6334	20232	0.098	R230310	3.2
<u>Totals</u>						
Unknowns		0	0	0.000		
Named		16045	46738	0.200		
Grand Total		16045	46738	0.200		

**APPENDIX 2**

**Typical Gas Chromatograms for ICIA5504 and R230310  
Residue Determination in Grapes**

- Figure 1: 1.0  $\mu\text{g cm}^{-3}$  ICIA5504 and R230310 standard.
- Figure 2: Untreated grape sample at 1.0  $\text{g cm}^{-3}$ .
- Figure 3: Untreated grape sample at 1.0  $\text{g cm}^{-3}$  fortified at 1.0  $\text{mg kg}^{-1}$ .  
Recovery = 105% ICIA5504, 111% R230310.

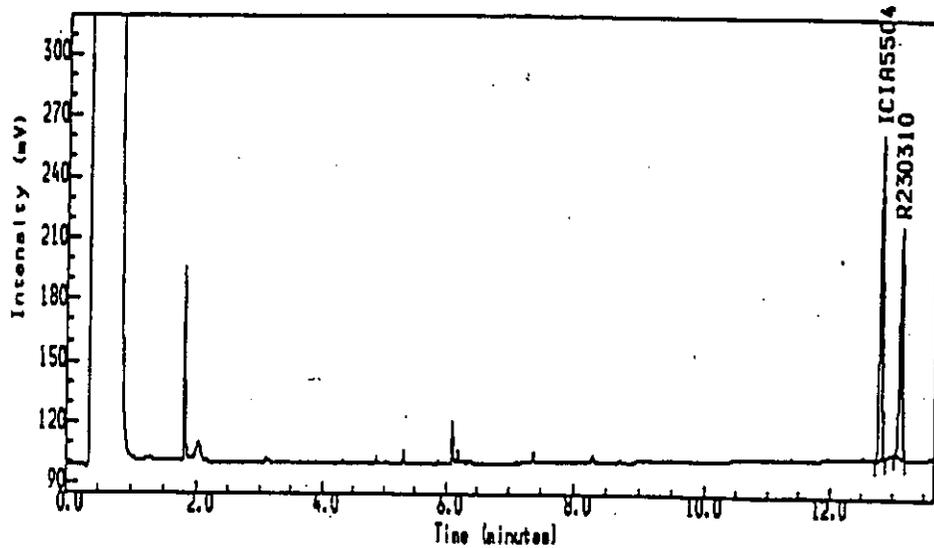
Figure 1

[RESIDUE] 16 AS6993A,13,1  
 Reported on 16-MAY-1995 at 14:32

---

Injection Report

Acquired on 4-MAY-1995 at 22:02



Sample Name : D9579/35B  
 Sample Id : 6993/95/13  
 Sample Type : Standard Amount=1.00000  
 Bottle No : 1

PEAK INFORMATION

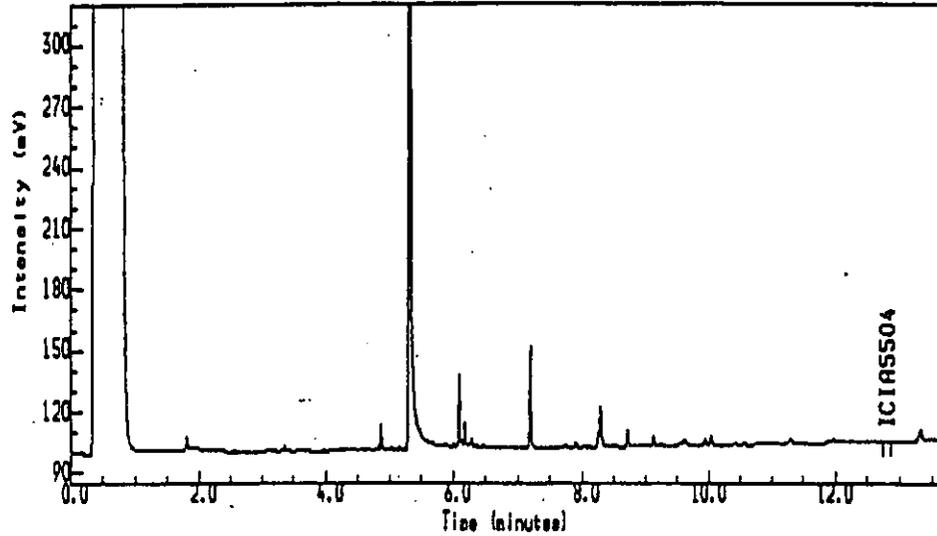
Peak	RT mins	Hgt uV	Area uVs	MG/KG	Peak name	Width
1	12.755	158276	430368	1.004	IC1A5504	2.9
2	13.115	112151	340504	0.977	R230310	3.2
<u>Totals</u>						
Unknowns	0	0	0	0.000		
Named	270427	770871	1.981			
Grand Total	270427	770871	1.981			

Figure 2

[RESIDUE] 16 AS6993A,10,1  
 Reported on 16-MAY-1995 at 14:34

Injection Report

Acquired on 4-MAY-1995 at 20:38



Sample Name : 468/7/1 95  
 Sample Id : 6993/95/10  
 Sample Type : Control Amount=1.00000  
 Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Hght uV	Area uVs	MG/KG	Peak name	Width
1	12.795	427	1092	0.003	ICI85504	2.9
<b>Totals</b>						
Unknowns		0	0	0.000		
Named		427	1092	0.003		
Grand Total		427	1092	0.003		

ANALYSIS SUMMARY

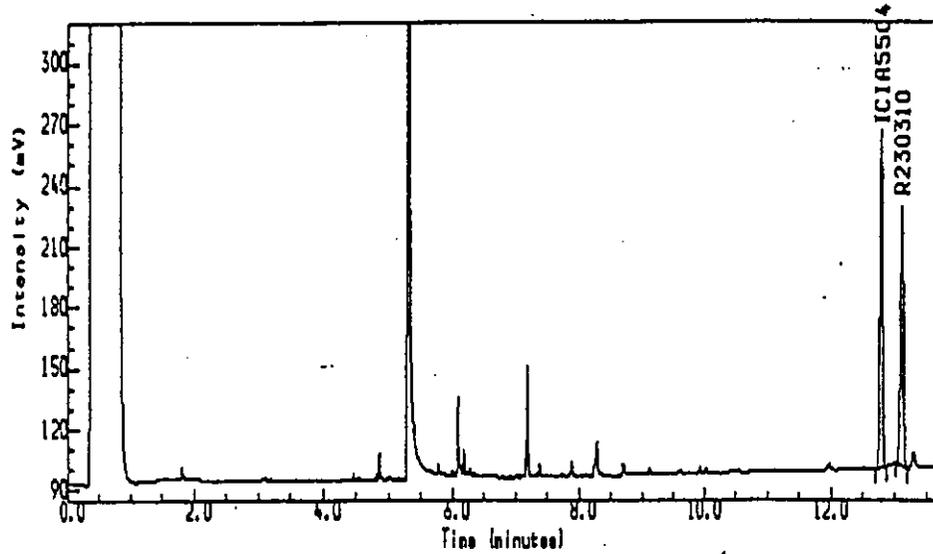
Method..... DMC5504  
 Run sequence..... DMK6993  
 Calibration..... DMC5504  
 External standard calibration using height

Figure 3

[RESIDUE] 16 AS6993A,11,1  
 Reported on 16-MAY-1995 at 14:33

Injection Report

Acquired on 4-MAY-1995 at 21:06



Sample Name : R1 468/8/1 95  
 Sample Id : 6993/95/11  
 Sample Type : Recovery Amount=1.00000  
 Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Hgt W	Area U/S	MG/KG	Peak name	Width
1	12.789	166333	459174	1.055	IC1A5504	2.9
2	13.109	127813	381991	1.114	R230310	3.2
<u>Totals</u>						
Unknowns	0	0	0	0.000		
Named	294146	841165	2.168			
Grand Total	294146	841165	2.168			

**APPENDIX 3**

**Materials/Safety**

## 1 Apparatus

- a) Glass centrifuge bottles (250 cm<sup>3</sup> capacity) for sample extraction.
- b) High speed homogeniser, eg. Sorval Omni-Mixer.
- c) Filtration apparatus: Büchner funnel, adapter, filter paper (Whatmar)
- d) Round bottom flasks (250, 100 cm<sup>3</sup> capacity).
- e) Glass filter funnels (5 cm diameter)
- f) Rotary evaporator e.g Büchi
- g) Vacuum manifold system eg. Supelco<sup>™</sup> for solid phase extraction.
- h) Glass reactivials (7 cm<sup>3</sup> capacity).
- i) Vials for GC and HPLC analysis.
- j) A gas chromatograph fitted with a nitrogen phosphorus detector eg. Varian 3400 series, autosampler and integrator or data handling system.
- k) HPLC system with a UV detector, autosampler and integrator or data handling system.

## 2 Reagents

- a) Solvents: acetone, acetonitrile, ethyl acetate, hexane and dichloromethane, Fisher Scientific HPLC grade or equivalent.
- b) Solid phase extraction sorbents (Si) available from Analytichem International Inc.
- c) Florisil<sup>™</sup> (100-200 US mesh) for chromatographic use available from Fisher Scientific.
- d) Granular anhydrous sodium sulphate (ACS reagent). Heat in an oven at 140°C for 24 hours to remove volatile contaminants.
- e) Glass wool - contaminants are removed by soaking the glass wool overnight in hexane (in a fume cupboard), allowing hexane to fully evaporate and placing in a oven at 100°C for 24 hours.
- f) GC capillary columns:
  - Rt<sub>x</sub>- 1 (100% dimethyl polysiloxane, OV-1, DB-1, RSL-150 equivalent) fused silica wall coated open tubular megabore 15 m x 0.53 mm internal diameter (1 µm film thickness).
  - Rt<sub>x</sub>- 200 (trifluoropropylmethyl polysiloxane) fused silica wall coated open tubular megabore 30 m x 0.53 mm internal diameter (1 µm film thickness) or capillary 15 m x 0.32 mm internal diameter (0.5 µm film thickness).  
(Rt<sub>x</sub> columns available from Thames Chromatography, Maidenhead, Berkshire, England, UK).
- g) HPLC column, Spherisorb<sup>™</sup> 5 ODS2 25 cm x 4.6 mm internal diameter, available from Phenomenex USA, 2320 W 205th Street, Torrance, California 90501.

- h) A sample of ICIA5504 and R230310 of known purity, available from Zeneca Agrochemicals, UK.

### 3 Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate safety manual (e.g. Zeneca Laboratory Safety Manual) which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by G D Muir, The Chemical Society, London.

#### a) Solvent Hazards

	Acetone	Ethyl acetate	Acetonitrile	Hexane	Dichloromethane
Harmful vapour	Yes	Yes	Yes	Yes	Yes
Highly flammable	Yes	Yes	Yes	Yes	No
Harmful by skin absorption	No	Yes	Yes	No	No
TLV mg/m <sup>3</sup>	2400	1400	70	180	350

In all cases avoid breathing vapour. Avoid contact with skin and eyes.

- b) ICIA5504 has a divisional toxicity class of 4. ICIA5504 has a mammalian toxicity (acute oral LD<sub>50</sub>) in rat greater than 5000 mg kg<sup>-1</sup>.

### 4 Preparation of Analytical Standards

Weigh out accurately using a five figure balance, sufficient of ICIA5504 and R230310 solid to allow dilution in acetone to give a 1000 µg cm<sup>-3</sup> stock solution in a volumetric flask. Make serial dilutions from the stock to give 100 µg cm<sup>-3</sup> standard solution. Prepare 10 µg cm<sup>-3</sup>, 1.0 µg cm<sup>-3</sup> and 0.1 µg cm<sup>-3</sup> mix standard solutions of ICIA5504 and R230310 in acetone to be used for fortification of recovery samples.

When not in use, always store the standard solutions, securely stoppered, in a refrigerator at ≤7°C to prevent decomposition and/or concentration of the solvent strength. Analytical standards should be freshly prepared from the solid material after six months of use.